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## *In vitro* and *in vivo* Anti-plasmodial Activities of *Gynura procumbens*

(Kajian *in vitro* dan *in vivo* Aktiviti Anti-plasmodium *Gynura procumbens*)

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### ABSTRACT

*Gynura procumbens*, locally known in Malaysia as Sambung Nyawa is a medicinal plant belonging to the Asteraceae (Compositae) family. *G. procumbens* have been traditionally used by the local and indigenous populations to treat an array of ailments ranging from skin conditions and fever to kidney disease, inflammation and diabetes. In the present investigation, aqueous and ethanol extracts of *G. procumbens* were evaluated for anti-plasmodial activities *in vitro* and *in vivo*. Survival of two chloroquine-sensitive strains of malarial parasites; rodent *Plasmodium berghei* NK65 and human *Plasmodium falciparum* 3D7 was determined following incubations *in vitro* with extracts. Based on parasite lactate dehydrogenase (pLDH) assay, both extracts were shown to inhibit parasite proliferation to varying degrees. The aqueous extract was more potent than the ethanol extract at suppressing growth of both parasites *in vitro*; each displaying  $IC_{50}$  values of  $12.40 \pm 6.02$  and  $14.38 \pm 7.53$   $\mu\text{g/mL}$  towards *P. berghei* NK65; and  $25.69 \pm 4.34$  and  $42.23 \pm 7.19$   $\mu\text{g/mL}$  towards *P. falciparum* 3D7, respectively. The aqueous extract was found to be selective for *P. falciparum* (Selectivity Index 64.30). Four-day suppressive tests in ICR mice showed dose-dependent chemo-suppressive activities of both plant extracts tested towards *P. berghei* NK65. Daily intra-peritoneal injections of the aqueous extract of *G. procumbens* at 25, 50 or 100 mg/kg for four consecutive days showed chemo-suppression of  $50.42 \pm 3.17$ ,  $65.95 \pm 5.48$  and  $81.92 \pm 3.07\%$ , respectively. At the same dosages, the ethanol plant extract resulted in  $44.97 \pm 3.44$ ,  $55.21 \pm 3.87$  and  $64.44 \pm 4.05\%$  chemo-suppression respectively. At 250 mg/kg/day, only the aqueous plant extract gave >90% chemo-suppression ( $93.06 \pm 5.46\%$ ). Treatment of *P. berghei*-infected mice with extracts improved the median survival time compared with non-treated infected mice. This represents the first report showing anti-plasmodial activity of *G. procumbens*.

**Keywords:** Anti-plasmodial; Asteraceae; *Gynura procumbens*; *in vitro*; *in vivo*

### ABSTRAK

*Gynura procumbens*, yang lebih dikenali di Malaysia sebagai pokok Sambung Nyawa, merupakan tumbuhan ubatan daripada famili Asteraceae (Compositae). *G. procumbens* digunakan secara tradisi oleh penduduk tempatan termasuk Orang Asli untuk merawat jurang penyakit yang luas iaitu daripada penyakit kulit dan demam ke penyakit ginjal, inflamasi dan diabetes. Dalam kajian ini, ekstrak akueus dan etanol *G. procumbens* dinilai untuk aktiviti anti-plasmodium secara *in vitro* dan *in vivo*. Kemandirian dua strain parasit malaria sensitif-klorokuin iaitu strain roden *Plasmodium berghei* NK65 dan strain manusia *Plasmodium falciparum* 3D7 ditentukan selepas eraman *in vitro* bersama ekstrak. Berdasarkan pengasaan laktat dehidrogenase plasmodium (pLDH), kedua-dua ekstrak didapati merencat proliferasi parasit dengan perkadaran berbeza. Ekstrak akueus mempamerkan potensi perencatan parasitemia *in vitro* lebih baik berbanding dengan ekstrak etanol; setiap satu mempamerkan nilai  $IC_{50}$   $12.40 \pm 6.02$  dan  $14.38 \pm 7.53$   $\mu\text{g/mL}$  terhadap *P. berghei* NK65; dan  $25.69 \pm 4.34$  dan  $42.23 \pm 7.19$   $\mu\text{g/mL}$  terhadap *P. falciparum* 3D7. Ekstrak akueus didapati bersifat memilih terhadap *P. falciparum* (Indeks Pemilihan 64.30). Ujian kemo-penekanan empat hari dalam mencit ICR menunjukkan aktiviti kemo-penekanan terhadap *P. berghei* NK65 yang berkadar dos bagi kedua-dua ekstrak tumbuhan yang diuji. Penyuntikan ekstrak akueus *G. procumbens* secara intra-peritonium selama empat hari berturut-turut ke atas mencit terinfeksi-*P. berghei* NK65 pada dos 25, 50 dan 100 mg/kg/hari masing-masing menunjukkan kemo-penekanan  $50.42 \pm 3.17$ ,  $65.95 \pm 5.48$  dan  $81.92 \pm 3.07\%$ . Pada dos yang sama, ekstrak etanol masing-masing menyebabkan kemo-penekanan  $44.97 \pm 3.44$ ,  $55.21 \pm 3.87$  dan  $64.44 \pm 4.05\%$ . Pada dos 250 mg/kg/hari, hanya ekstrak akueus memberi kemo-penekanan >90% ( $93.06 \pm 5.46\%$ ). Perlakuan ekstrak akueus kepada mencit terinfeksi-*P. berghei* juga mampu meningkatkan median tempoh kemandirian mencit berbanding mencit terinfeksi tanpa perlakuan ekstrak. Penemuan ini merupakan laporan pertama mengenai aktiviti anti-plasmodium *G. procumbens*.

**Kata kunci:** Anti-plasmodium; Asteraceae; *Gynura procumbens*; *in vivo*; *in vitro*

### INTRODUCTION

*Gynura procumbens*, a medicinal plant belonging to the Asteraceae (Compositae) family locally known in Malaysia

as the *Sambung nyawa* plant, is commonly found growing wild or cultivated in various parts of South-east Asia, especially Malaysia, Indonesia and Thailand (Bhore et al.

2010). Traditionally, preparations from the plant have been used among others as poultices or boiled extracts to treat ailments ranging from skin conditions and fevers to kidney disease, inflammation and diabetes (Perry 1980). Even the leaves of the plant are eaten raw for overall health.

The scientific basis for the traditional uses of *G. procumbens* has been especially extensively investigated in diabetes. Anti-hyperglycaemic activities displayed by various extracts of *G. procumbens* in experimentally-induced diabetic animals (Akowuah et al. 2001, 2002) have been attributed to components found in the plant concoction including flavonoids, saponins, tannins, terpenoids and sterol glycosides (Akowuah et al. 2002), rutin, kaempferol, kaempferol-3-O-rutinoside (quercetin) and astragalin (Zurina et al. 2010). In addition, anti-hyperlipidemic (Zhang & Tan 2000) and anti-inflammatory (Iskander et al. 2002) activities associated with *G. procumbens* have also been documented.

Interestingly, the current first-line anti-malarial drug, artemisinin was originally derived from *Artemisia annua* also belonging to the Asteraceae family. Plants from the same family also reported to exhibit anti-plasmodial activities include *Tithonia diversifolia* (Elufioye & Agbedahunsi 2004), *Anisopappus chinensis* (Gathirwa et al. 2007) and *Artemisia afra* (Lusakibanza et al. 2010). Collectively, reports on the anti-inflammatory properties of *G. procumbens* (Iskander et al. 2002) and the ability of the Plasmodium parasite to elicit inflammatory responses (Kaur et al. 2009) as other pathogenic agents thus merit the evaluation of *G. procumbens* for anti-plasmodial activities.

Additionally, this investigation represents another effort to screen our natural tropical heritage for alternative therapeutics against malaria, the need of which is primarily linked to resistance development of the parasite to current drugs.

## MATERIALS & METHODS

### PLANT MATERIALS

*G. procumbens* plants were collected from the herbal experimental plot at the Faculty of Science and Technology, Universiti Kebangsaan Malaysia. Fresh leaves were washed, air-dried at room temperature and then ground into a powder form before maceration with 95% ethanol for three cycles; each cycle involving three day soaking at room temperature. The extracts were filtered and concentrated using a rotary evaporator (Buchi, Switzerland) under reduced pressure at 40°C to yield a concentrated ethanol extract. The aqueous extract was prepared by further soaking of the residue from the previous filtration step in ultra-pure water for 24 h and filtered again. The plant extracts were freeze-dried (Labconco, USA) and stored dry in a refrigerator at 4°C until used for further experiments.

### PREPARATION OF *P. berghei* INFECTED RED BLOOD CELLS

Mice were infected with parasites ( $1 \times 10^6$  *P. berghei* NK65 infected erythrocytes) through the intra-peritoneal route and parasitaemia monitored daily in thin blood smear slides prepared from tail blood. When 2-3% was attained, blood was withdrawn via cardiac puncture and washed with culture medium to get rid of platelets and lysed red blood cells. This processed blood was then directly used for pLDH assay. Blood from normal non-infected mice was used as negative control.

### IN VITRO *P. falciparum* CULTURE

The human parasite *P. falciparum*, strain 3D7 was obtained from The Malaria Research and Reference Reagent Resource Center (MR4) and maintained in continuous parasite culture according to methodology described by Trager and Jensen (1976). The culture was carried out using complete medium in T25 culture flasks and incubated at 37°C, 3% O<sub>2</sub>, 5% CO<sub>2</sub> and 91% N<sub>2</sub>. Parasitaemia was maintained so that less than 10% of the red blood cells used in culture were infected. Non-infected human blood group O Rh-positive erythrocytes (<28 days old) served as host cells. The medium was changed every 24 h and blood smear slides prepared every 48 h to monitor parasitaemia levels.

### IN VITRO ANTI-PLASMODIAL ASSAY

Plant extracts were assessed for anti-plasmodial activity *in vitro* against chloroquine-sensitive strains of *P. falciparum* 3D7 and *P. berghei* NK65, using a modified parasite lactate dehydrogenase (pLDH) method (Nkhoma et al. 2007). Crude plant extracts were first dissolved in culture medium at concentrations of 1 µg/mL and then further diluted to 8 different concentrations (ranging from 0.0001 to 100 µg/mL). Chloroquine diphosphate (Sigma Chemical, USA) were dissolved in culture medium (1 µg/mL) and served as controls in all experiments. All tests were performed in triplicate. Asynchronous cultures with parasitaemia of 2-3% and a final haematocrit of 1.5% were aliquoted into flat bottom 96-well microtiter plates and incubated at 37°C for 48 h (for *P. falciparum*) or 24 h (*P. berghei*). After the incubation period, the plates were frozen at -20°C overnight, followed by three thaw-freeze cycles to lyse the red blood cells. At the end of thaw-freeze cycles, 100 µL of Malstat reagent and 25 µL of Nitroblue tetrazolium/Phenazine ethosulphate (NBT/PES) solution were added to each well of a new flat bottomed 96-well microtiter plate. The culture in each of the wells of the original plate was resuspended by mixing with a multichannel pipette. Then, 15 µL of the culture was taken from each well and added to the corresponding well of the Malstat plate, thereby initiating the lactate dehydrogenase reaction. Color development of the LDH plate was monitored colorimetrically at 650 nm with the aid of a plate reader after an hour of incubation in the dark using microplate reader (Optima, Germany). IC<sub>50</sub>

values were calculated using HN-NonLin Software (Noedl 2002). The anti-plasmodial activities of the test extracts were expressed as  $IC_{50 \text{ pLDH}}$  (mean  $\pm$  S.D. of at least three separate experiments performed in triplicate).

#### CYTOTOXICITY ASSAY

The cytotoxicity of both test extracts were assessed by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay (Kayano et al. 2011) to evaluate their effects on proliferation of Chang liver cells. Cells were cultivated in DMEM medium supplemented with 10% heat-inactivated foetal bovine serum, penicillin (1 unit/mL) and streptomycin (1 unit/mL) in a humidified atmosphere of 5%  $CO_2$  at 37°C. Chang cells were seeded in 96-well plates at a density of  $5 \times 10^4$  cells per well and incubated for 24 h to allow cell adherence. Incubation of cells with extracts were carried out by removing the culture media from adhered liver cells and replacing this with 100  $\mu$ L of extract at different concentrations (0.001-10000 mg/mL) or with the same volume of culture media for controls.

After 48 h of incubation under the same conditions described above, the mixture in each well was removed; replaced with 100  $\mu$ L culture media and 10  $\mu$ L of MTT solution (5 mg/mL in PBS) and further incubated for 3 h. The formazan crystals produced by viable cells in each well were resuspended in 100  $\mu$ L of DMSO and  $A_{590}$  readings determined using an ELISA reader (Microplate Reader BIO-RAD Model 680, USA).  $IC_{50}$  values were determined using non-linear regression (Becker et al. 2011) and cytotoxicity activities expressed as  $IC_{50 \text{ cytotoxicity}}$ .

#### SELECTIVITY INDEX

Selectivity Index (SI), corresponding to the ratio between anti-plasmodial and cytotoxic activities, was calculated for each test extract according to the following formula (Hout et al. 2006):

$$\text{Selectivity Index, SI} = IC_{50 \text{ Cytotoxicity}} / IC_{50 \text{ pLDH}}.$$

#### ADAPTATION OF ANIMALS

Male ICR mice aged seven weeks and weighing approximately 30 g were obtained from the Animal House facility at Universiti Kebangsaan Malaysia. The mice were housed in cages and maintained on standard laboratory rat chow with water ad-libitum. All mice were acclimatised for two weeks before the initiation of the experimentation. Permission and approval for animal studies were obtained from the Universiti Kebangsaan Malaysia Animal Ethics Committee (UKMAEC).

#### TOXICITY TEST

Toxicity experiments were carried out as described by Dikasso et al. (2006) with minor changes. Separate experiments were carried out for each test extract. Four groups of mice ( $n=7$  per group) were intra-peritoneally

injected with extracts at four different dosages (25, 50, 100 and 250 mg/kg/day); similar dosages were employed in the four-day suppressive test. The control group of mice was given 0.9% saline.

Mice were observed for gross physical and behavioural changes. Survival was monitored for 30 days. Mortality occurring before day 5 post-treatment with extract represents toxicity in the experimental animals (Hilou et al. 2006).

#### IN VIVO ANTI-PLASMODIAL ASSAY

The anti-plasmodial assay was performed based on the method described by Peters and Robinson (1992). Male ICR mice (6 weeks old,  $n=35$ ) were injected intra-peritoneally with an inoculum of  $1 \times 10^6$  *P. berghei* NK65-infected erythrocytes at the commencement of the experiment (day 0). The inoculum was prepared by diluting infected stock mice blood at 20-30% parasitaemia with Alsever's solution.

Groups of seven infected mice were then administered with test extracts (25, 50 and 100 mg/kg/day) daily for four consecutive days by intra-peritoneal injection. Chloroquine diphosphate (10 mg/kg/day) and saline 0.9% were similarly administered in control groups, respectively. Parasitaemia (%) was determined daily in tail blood of the animals as described previously (Nurul Aiezzah et al. 2010). On the fifth day after administration of parasite inoculum (day 4), percentage chemo-suppression caused by the test extracts was calculated by comparing parasitaemia present in infected controls with those of test mice treated with the extracts (Elufioye & Agbedahunsi 2004). Separate experiments were carried out for each extract and also for testing the 250 mg/kg/day dosage of both extracts. For all groups of mice, survival time in days was recorded and median survival time determined for each group.

#### STATISTICAL ANALYSIS

For *in vitro* experiments, the average  $IC_{50}$  value was obtained by non-linear regression using HN-NonLin Software (Noedl 2002). For *in vivo* experiments, all results were expressed as the mean  $\pm$  standard deviation in each treatment group. Statistical analysis was performed using Anova single factor in Microsoft Excel 2003 for significance of difference between two groups. Animal survival graphs were plotted and median survival time was determined following Kaplan Meier analysis using Graph Pad Prism 5.

#### RESULTS

##### IN VITRO CYTOTOXICITY ASSAY

MTT assay performed using Chang liver cells showed no significant cytotoxic activity associated with the aqueous extract of *G. procumbens* with an  $IC_{50}$  value exceeding 1000  $\mu$ g/mL. However, the ethanol extract

exhibited mild toxicity towards these normal mammalian cells ( $IC_{50}$  100-500  $\mu\text{g/mL}$ ) (Ramazani et al. 2010).

#### IN VITRO ANTI-PLASMODIAL ASSAY

Anti-plasmodial activities of both aqueous and ethanol plant extracts against chloroquine-sensitive *P. falciparum* 3D7 and *P. berghei* NK65 determined using pLDH assay on parasites incubated *in vitro* (37°C, 5%  $\text{CO}_2$ ) revealed  $IC_{50}$  values  $\leq 50 \mu\text{g/mL}$  (Table 1). Both aqueous and ethanol extracts of the plant therefore displayed active anti-plasmodial activities as categorised by Ramazani et al. (2010). Further comparison of toxicity of tested extracts towards Chang liver cells with their respective anti-plasmodial (*P. falciparum* 3D7) activity gave a SI value of 64.30 for the aqueous extract exceeding 10 which is the acceptable cut-off index for therapeutic consideration (Tamez et al. 2005). Thus, the *G. procumbens* aqueous extract selectively suppressed proliferation of the human malarial parasite strain *in vitro* with no effect on normal mammalian cells.

#### IN VIVO TOXICITY TEST

Mice administered daily with intra-peritoneal injections of the aqueous extract of *G. procumbens* at 25, 50, 100 or 250 mg/kg/day for four days survived till day 30 post-injection of extract. No signs of toxicity such as diarrhoea,

excess urination and lethargy were observed in the study animals.

#### FOUR-DAY SUPPRESSIVE TEST

Four-day suppressive tests revealed that both aqueous and ethanol extracts of *G. procumbens* caused dose-dependent inhibition of parasitaemia development in malaria-infected mice (Table 2). Repetitive intra-peritoneal injections of 25, 50 and 100 mg/kg/day aqueous extract of *G. procumbens* for four consecutive days into *P. berghei* NK65-infected mice resulted in  $50.42 \pm 3.17$ ,  $65.95 \pm 5.48$  and  $81.92 \pm 3.07\%$  suppression of parasitaemia, respectively, compared with non-treated control infected mice. Administration into mice with the anti-malarial reference drug, chloroquine (10 mg/kg/day) caused 100% chemo-suppression. Similar chemo-suppressive activity was observed in *G. procumbens* ethanol extract-injected mice with  $44.97 \pm 3.44$ ,  $55.21 \pm 3.87$  and  $64.44 \pm 4.05\%$  for 25, 50 and 100 mg/kg/day dosages, respectively. At the end of the experimental period of 28 days, none of the ethanol extract-treated infected mice survived (Figure 1). There were however, surviving mice on day 28 for the aqueous extract-treated group of mice (Figure 2). Non-treated parasite-infected animals did not survive beyond day 20.

Four-day suppressive test data indicated that from as low as 50 mg/kg/day dosage, the aqueous *G. procumbens* extract was capable of inhibiting *P. berghei* parasitaemia

TABLE 1. Anti-plasmodial activity of aqueous and ethanol extracts of *G. procumbens* against chloroquine-sensitive *P. falciparum* 3D7 and *P. berghei* NK65 compared with chloroquine

Extract	Average $IC_{50}$ value Chang liver cells ( $\mu\text{g/mL}$ )	Average $IC_{50}$ value <i>P. berghei</i> NK65 ( $\mu\text{g/mL}$ )	Average $IC_{50}$ value <i>P. falciparum</i> 3D7 ( $\mu\text{g/mL}$ )	Selectivity Index
<i>G. procumbens</i> (Aq)	$1652.55 \pm 7.83$	$12.40 \pm 6.02$	$25.69 \pm 4.34$	64.30
<i>G. procumbens</i> (EtOH)	$424.20 \pm 5.62$	$14.38 \pm 7.53$	$42.23 \pm 7.19$	10.05

The results are based on average  $IC_{50} \pm \text{SD}$  ( $\mu\text{g/mL}$ ) values from 3 replicates ( $n=3$ ) for *in vitro* cytotoxicity activity and anti-plasmodial activity

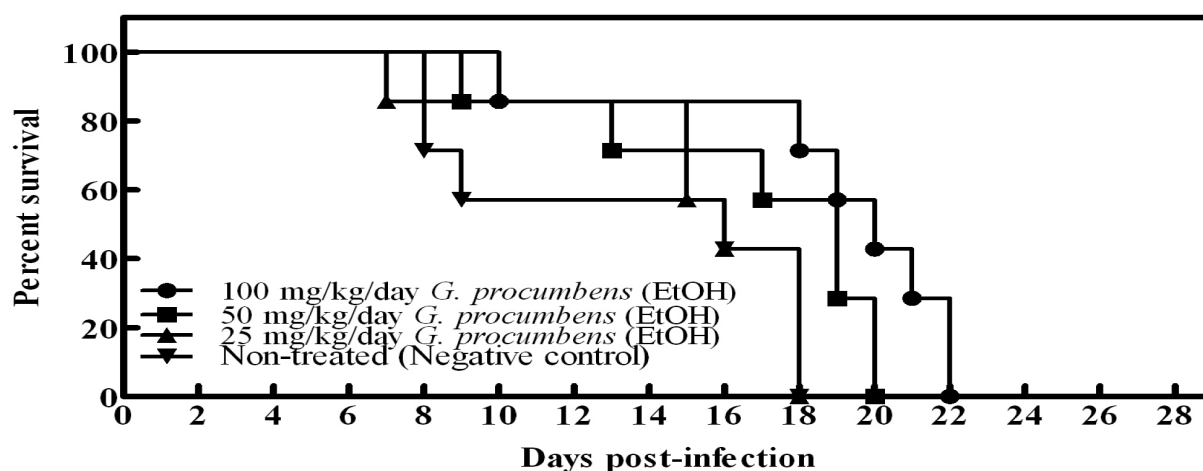
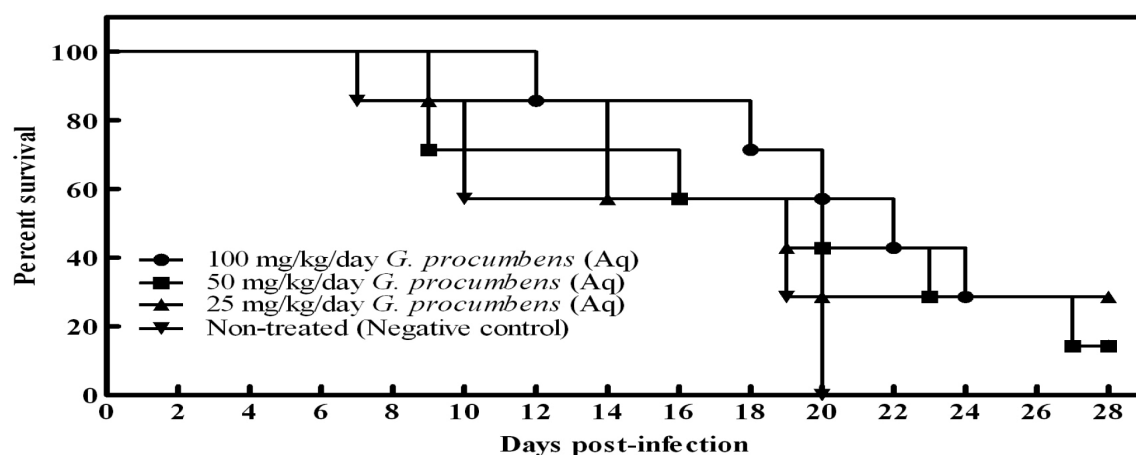
TABLE 2. Effects of *G. procumbens* aqueous and ethanol extracts on malarial infection

Drug/extract	Dosage (mg/kg/day)	Average parasitaemia inhibition on day 4 (%)	Median survival time (days)
<i>G. procumbens</i> (Aq)	100	$*81.92 \pm 3.07$	22
	50	$*65.95 \pm 5.48$	20
	25	$*50.42 \pm 3.17$	19
Chloroquine (Positive control)	10	100	28
0.9% Saline (Negative control)	0.1 mL	-	19
<i>G. procumbens</i> (EtOH)	100	$*64.44 \pm 4.05$	20
	50	$*55.21 \pm 3.87$	19
	25	$*44.97 \pm 3.44$	16
Chloroquine (Positive control)	10	100	28
0.9% Saline (Negative control)	0.1 mL	-	16

The results show chemo-suppression  $\pm \text{SD}$  (%) compared with negative control ( $n=7$ ).

The symbol \* shows significant value, ( $p<0.05$ )



FIGURE 1. Survival of infected mice treated with ethanol extract of *G. procumbens*FIGURE 2. Survival of infected mice treated with aqueous extract of *G. procumbens*TABLE 3. Effects of *G. procumbens* aqueous and ethanol extracts at high dosage of 250 mg/kg/day on malarial infection

Drug/extract	Dose (mg/kg/day)	Average parasitaemia inhibition on day 4 (%)	Median survival (days)
<i>G. procumbens</i> (Aq)	250	*93.06 $\pm$ 5.46	21
<i>G. procumbens</i> (EtOH)	250	*84.73 $\pm$ 3.18	21
Chloroquine (Positive control)	10	100	28
0.9% Saline (Negative control)	0.1mL	-	17

The results show chemo-suppression  $\pm$  SD (%) compared with negative control ( $n=7$ ).  
The symbol \* shows significant value, ( $p<0.05$ )

development by more than 60%. Therefore, the aqueous extract of *G. procumbens* may be categorised as displaying highly active anti-plasmodial activity. The ethanol extracts on the other hand, may be classified as having moderate anti-plasmodial activity (Gathirwa et al. 2007).

In a separate four-day suppressive test experiment using a higher dosage of 250 mg/kg/day extracts in parasite-infected mice, chemo-suppression exceeding 90%

(93.06  $\pm$  5.46%) was attained with the aqueous extract (Table 3). According to WHO, extracts displaying very high level of chemo-suppression (>90%) at 250 mg/kg/day may be recommended for potential therapeutic development. Chemo-suppressive activity of the ethanol plant extract (84.73  $\pm$  3.18%) was not as high as that for the aqueous extract. Infected mice treated with the aqueous extract also showed improved survival compared with ethanol extract-treated or non-treated infected animals (Figure 3).

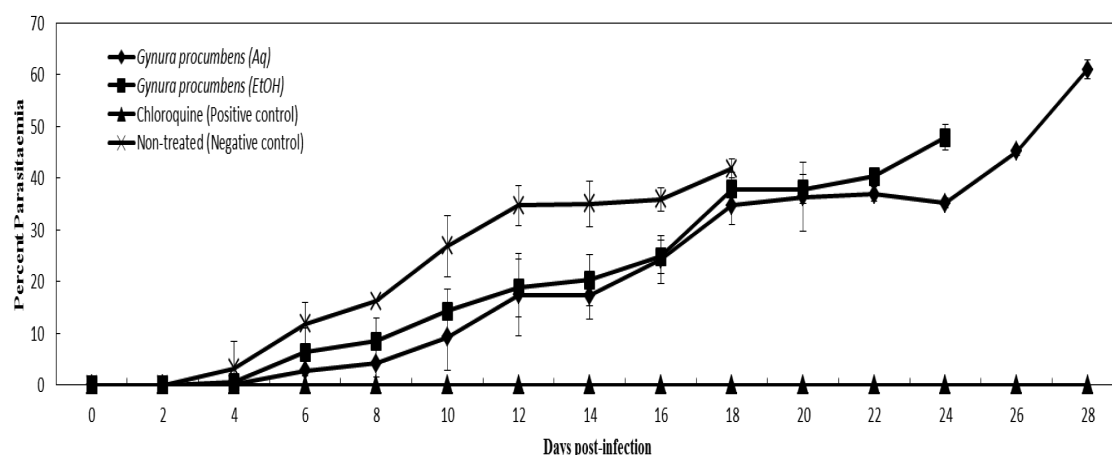


FIGURE 3. The development of *Plasmodium berghei* NK65 parasitaemia in ICR mice during treatment with 250 mg/kg/day of aqueous and ethanol extracts of *G. procumbens* given once daily for four consecutive days, from the first day of parasite injection

In terms of median survival time (Table 2), mice treated with aqueous *G. procumbens* extract displayed median survival times of 19, 20 and 22 days at dosages of 25, 50 and 100 mg/kg/day, respectively; versus 19 days for the non-treated group of study animals administered 0.9% saline. Median survival times for ethanol extract-treated mice were lower than that for the aqueous extract at 16, 19 and 20 days for the same three dosages. Non-treated infected mice given 0.9% saline displayed median survival time of 16 days.

## DISCUSSION

The traditional use of preparations from the *G. procumbens* plant to treat inflammatory-related conditions has been documented (Perry 1980). However, the scientific basis for the therapeutic claim has not been well-substantiated. To our knowledge, only one direct study on anti-inflammatory activity of *G. procumbens* has been reported (Iskander et al. 2002) and the chemical moiety responsible has not been identified.

Phytoconstituents identified in the plant have however, been associated with several bioactivities. For example, rutin, quercetin, kaempferol and astragalin were found to be responsible for blood glucose-lowering activity by mimicking or improving insulin action at the cellular level (Zurina et al. 2010). In our own laboratory, the anti-diabetic effect of a fraction from *G. procumbens* ethanolic extract (Chong et al. 2012) was most likely attributed to the kaempferol component (unpublished data). In addition, caffeoylquinic acid mixtures also present in the plant extract were linked with virucidal and anti-replicative activities against HSV-2 (Jiratchariyaku et al. 2000).

More importantly, quercetin and kaempferol have shown individual and synergistic effects against *P. falciparum* *in vitro* (Lehane & Saliba 2008). It is therefore likely that these two *G. procumbens* constituents contributed to the anti-plasmodial activities observed in

the present study i.e. for the aqueous plant extract and to a lesser extent, the ethanolic extract. The present finding that *G. procumbens* has potential anti-malarial properties is another beneficial element of this medicinal plant which to our knowledge has not been reported previously.

As in other pathogenic infections, plasmodial parasite invasion into its host triggers a series of events leading to inflammatory responses in the host to oust the invading microbe (Kaur et al. 2009). An important pathway involved in these responses is the phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) pathway (Yoo et al. 2005). Pivotal in mediating inflammatory responses is glycogen synthase kinase 3 (GSK-3) (Wang et al. 2011), a downstream component of the PI3K/Akt pathway. Initially identified for its role in glycogen metabolism (Embi et al. 1980), GSK-3 is now known to regulate a plethora of cellular processes (Chen et al. 2007).

Previously, we have shown that LiCl, as a direct inhibitor of GSK-3, suppressed parasitaemia development in a rodent malarial infection model (Nurul Aiezzah et al. 2010) thus implicating a role of this kinase in malarial pathogenesis. Interestingly, preliminary studies detected GSK-3-inhibitory activity in the aqueous extract prepared from *G. procumbens* absent in the ethanolic extract (unpublished data). This implicates the potential of the aqueous extract, like LiCl, to similarly affect *Plasmodium* parasite development via direct inhibition of GSK-3.

Coincidentally, kaempferol has been shown elsewhere to activate the PI3K/Akt pathway (Choi 2010; Lee et al. 2010). Therefore, the phytoconstituents in *G. procumbens* responsible for anti-plasmodial activities may act directly on GSK-3 as seen with LiCl or indirectly through PI3K/Akt as reported for kaempferol.

## CONCLUSION

Despite the availability of some scientific evidence for a range of biological activities associated with the

traditional phytotherapeutic uses of *G. procumbens*, the present study provides for the first time, data on the anti-plasmodial activity of *G. procumbens*, yet another plant in the Asteraceae family. The promising results obtained here especially with the aqueous extract of the plant warrants further fractionation to identify active principles responsible for the anti-plasmodial activity. In addition, whether this effect of *G. procumbens* may be due to the activation of PI3K/Akt pathway or due to direct inhibitory effect on GSK-3 remain to be addressed.

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